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Abbreviations

AhR: aryl hydrocarbon receptor; ARNT: aryl hydrocarbon receptor nuclear translocator; AT: adipose tissue; BDE: CVD: cardiovascular diseases; CYP: cyrtochrome P450; DDE: 1,1-Dichloro-2,2-bis(*p*-chlorophenyl)ethylene; DDT: dichlorodiphenyltrichloroethane; DL: dioxin-like; K_{ow}: octanol:water ratio partition coefficient; HDL: high density lipoprotein; MRI: magnetic resonance imaging; LD50: lethal dose; LDL: low density lipoprotein; NFκB: nuclear factor kappa-light-chain-enhancer of activated B cells; PBDE: Polybrominated diphenyl ethers ; PBPK: physiologically-based pharmacokinetic; PCB: polychlorinated biphenyl; PFOA: perfluorooctanoic acid; PFOS: perfluorooctane sulfonate; PND: postnatal day; POP: persistent organic pollutant; PPARγ: Peroxisome proliferator-activated receptor gamma; TCDD: 2,3,7,8-tetrachlorodibenzo-*p*-dioxin; VLDL: very low density lipoprotein; Vss: volume of distribution during steady state;

ABSTRACT

Background. Adipose tissue (AT) is involved in several physiological functions, including metabolic regulations, energy storage, and endocrine functions.

Objectives. The aim of this review is to analyze the evidence that an additional function of the AT is to modulate persistent organic pollutant (POP) toxicity through several mechanisms.

Methods. We have reviewed the literature on the interaction of AT with POPs in order to provide a comprehensive model for this additional function of the AT.

Discussion. As a storage compartment for lipophilic POPs, AT plays a critical role in the toxicokinetics of a variety of drugs and pollutants, in particular POPs. By sequestering the POPs, AT can protect other organs and tissues from POP overload. However, this protective function could prove to be a threat in the long run. The accumulation of lipophilic POPs will increase total body burden. These accumulated POPs are slowly released into the bloodstream, and more so during weight loss. Thus, AT constitutes a continual source of internal exposure to POPs. In addition to its buffering function, AT is also a target of POPs and may mediate part of their metabolic effects. This is particularly relevant as many POPs have been shown to display obesogenic effects leading to quantitative and qualitative alterations of AT. Some POPs also induce a proinflammatory state in the adipose tissue, which may lead to detrimental metabolic effects.

Conclusion. The AT appears to play diverse functions both as a modulator and as a target of POP toxicity.

Introduction

Obesity is increasingly frequent in developed countries and is a commonly known risk for disorders such as impaired glucose tolerance, metabolic syndrome, diabetes mellitus, liver and cardiovascular diseases (CVD), as well as cancer (Ludescher et al. 2009). Adipose tissue (AT) has historically been considered a simple storage tissue but its physiological functions have been considerably reassessed over the last decade (Lafontan 2012). Evidence for metabolic and endocrine AT functions has accumulated. Further knowledge of the histological architecture of AT as well as the role of AT stroma including immune cells has also been obtained. Greater attention is now given to the pathological contribution of AT to obesity and metabolic disorders such as type 2 diabetes. Lately, various interactions between AT and persistent organic pollutants (POPs) have been established suggesting that this tissue plays a significant role in the kinetics and the toxicity of POPs.

Based on these studies, we propose that, in addition to its other metabolic and endocrine functions, AT has an identified and diverse toxicological function. First, AT can store a variety of hydrophobic xenobiotic chemicals, in particular POPs. Second, AT also constitutes a low-grade internal source of stored POPs leading to continuous exposure of other tissues. Third, AT can be a target for the effects of a xenobiotic chemical which alters AT functions, increases AT inflammation, and/or modulates the differentiation of AT precursor cells. For instance, obesogens are exogenous chemicals (from a nutritional, pharmaceutical, or environmental origin) that directly or indirectly increase obesity through disruption of metabolic, hormonal, or developmental processes (Grun and Blumberg 2007; Schug et al. 2011). Conversely, several POPs are known to induce cachexia, particularly at high doses. In this review, we discuss these issues to present evidence that supports a complex, previously unsuspected, role of AT in toxicology.

METABOLIC AND ENDOCRINE FUNCTIONS OF AT

AT is classically viewed as the main reservoir of energy mobilized from the body. In fact, AT is not merely an energy depot, it is essential for normal carbohydrate and lipid homeostasis. When stimulated by insulin, adipocytes store glucose as triglycerides in lipid droplets (Stolic et al. 2002). Adipocytes meet the energy needs in states of metabolic stress such as fasting by releasing fatty acids through lipolytic processes (Lafontan 2012). In addition to its energy storing function, adipocytes secrete several endocrine factors such as leptin and adiponectin which regulate appetite as well as metabolic and inflammatory functions (reviewed in (Galic et al. 2010). The AT has substantial functional breadth in part due to the great diversity of cells within this tissue, such as adipocyte precursors (preadipocytes) in different states of differentiation, vascular cells, central nervous system cells, fibroblasts, and many immune cells. Beyond the adipocyte, AT is a site of storage and production of various substances with autocrine, paracrine, and neuroendocrine actions influencing behavior, energy regulation, lipid oxidation, immune and vascular function, hormonal status as well as its own metabolism and cellularity (reviewed in (Galic et al. 2010; Ouchi et al. 2011).

Together with adipocyte hypertrophy, obesity is characterized by the accumulation of macrophages in AT depots. Accumulation of macrophages in the visceral AT depot, but not the subcutaneous depot, is associated with liver injury (Tordjman et al. 2012). *In vitro* experiments have shown that macrophage secretions profoundly perturb adipose cell biology promoting a proliferative, proinflammatory, and profibrotic state of preadipocytes as well as an insulin resistant state of mature adipocytes. Lymphocytes, natural killer cells, and mast cells are found in AT parenchyma in obese people, and also in fibrosis depots which accumulate in obese subjects (Divoux et al. 2010).

The AT is much more than just an energy storehouse for the body or a repository for lipophilic chemicals. Obesity affects not only AT structure but its function. Thus because of

all these critical AT functions, the interaction of POPs with AT could lead to important metabolic and endocrine disruption.

AT AS A MECHANISM OF PROTECTION

Evidence of a protective function of AT. One of the most critical survival functions in a complex chemical environment is the ability of cells and organisms to detoxify and eliminate xenobiotic chemicals. The best studied detoxification machinery is the xenobiotic metabolizing system which includes receptors, metabolizing enzymes, and transporters, and which tends to prevent absorption, increase water solubility, or decrease reactivity of xenobiotic chemicals thus leading to their detoxification and elimination from the body (Barouki 2010). However, POPs are an important class of xenobiotic chemicals that are resistant to metabolism. POPs are environmentally and biologically persistent leading to their bioaccumulation and biomagnification up the food chain. Fatty foods of animal origin, e.g., meat, fish, and dairy, are known important vectors of several classes of POPs, including dioxins and PCBs (Bergkvist et al. 2008). POPs include certain organochlorine pesticides, polyhalogenated dibenzo-*p*-dioxins, furans, and biphenyls, as well as certain polybrominated flame retardants and perfluorinated chemicals. POPs do not readily undergo degradation by xenobiotic metabolizing enzymes, largely because of their high degrees of halogenation. However, some bind, often with high affinity, to certain xenobiotic receptors, as well as to certain xenobiotic metabolizing enzymes such as CYP1A2, without undergoing catalytic transformation (Diliberto et al. 1999). Such binding plays a significant role in their distribution as will be discussed below. Because of their hydrophobicity, POPs tend to distribute into lipophilic compartments, particularly the AT.

POPs are taken up by adipocytes and localize within lipid droplets (Bourez et al. 2012). However the precise location of POPs and their actual effects at the subcellular level

are poorly understood. It is believed that their accumulation within AT decreases their availability to other cells and tissues, thereby limiting their systemic toxicity. Experimental evidence supports such a protective function for AT. Studies conducted in the 1980s and 1990s showed that there was an inverse correlation between toxicity of POPs and fat mass of different animal species. Investigators compared the 30- day toxicity of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) in approximately twenty terrestrial animal species and found a positive correlation between the adiposity of these species and their LD50 (Geyer et al. 1993). These authors concluded that the species with the highest fat mass tended to display more resistance to TCDD in this acute toxicity test. Their conclusions were in line with studies showing that resistance of aquatic species to dioxin was also related to their fat mass content (Lassiter and Hallam 1990). However, these observations should not be taken as evidence that adiposity is the only factor discriminating dioxin- sensitive and resistant species. There is clear evidence for a major contribution of the genetics of the aryl hydrocarbon receptor (AhR) to dioxin toxicity.

The toxicokinetic role of AT. AT plays a major role in the storage and toxicokinetics of POPs because of their lipophilicity. The histological and anatomical structure of different ATs can influence their contribution to toxicokinetics. Recently, Sbarbati et al. (Sbarbati et al. 2010) proposed a new AT classification, based on AT organization, structure, surrounding tissue and anatomical localization. It is possible that different properties of these AT subtypes could have a pharmacokinetic impact on POPs and this will have to be further studied. However, there is currently no evidence for differences in POP content under steady state conditions between different types of AT (Kim et al. 2011).

Despite the presence of a large number of AT cell types, it is believed that POP storage in AT is primarily in the adipocytes. Adipocyte cytoplasm is almost totally composed of triglyceride droplets (Sbarbati et al. 2010). The transfer of POPs from the vascular

environment into the cell or through other tissue structures implies several pharmacokinetic factors such as tissue volume, anatomical localization, and blood flow rate influence the distribution of the chemicals into AT. The default approach is to assume that the tissue is flow limited, which means that the distribution of chemicals contained in blood across the well-stirred tissue compartment is fast and homogenous. Although this assumption is valid for the distribution of many xenobiotic chemicals into many tissues or organs, it appears to be incorrect for the movement of several highly lipophilic POPs across the AT due to their diffusion limited characteristics (also called permeability limited). In this case, the distribution of the chemicals is slower and may be incomplete. The physical basis of this AT diffusion limitation is related to the octanol:water ratio (K_{ow}) partition coefficient. The diffusion limitation is related to the exchange rate between the blood and adipose lipid, which becomes rate limiting if K_{ow} partition coefficient is large enough (Levitt 2010). In addition, the diffusion limitation values take into account the thickness and diameter of the adipose capillary network, and diffusion across the membrane. At steady state, the log K_{ow} predicts the capacity of the chemical to diffuse into AT and accumulate.

The best prospective mathematical pharmacokinetic method to estimate diffusion coefficients in AT is physiologically based pharmacokinetic (PBPK) modeling (Emond et al. 2006). Using PBPK modeling, we assumed that the diffusion coefficient parameter was constant across AT for a rate ranging between 4.5% to 5.0% of the cardiac output of 5.60 liters/minute (Derelanko and Hollinger 1995). However, as previously described, the rate of diffusion in AT is usually lower than that, resulting in a delay to reach steady state between blood and AT (Levitt 2010). In the future, we may need to determine these parameters in different AT types as there is some evidence that the rate of diffusion may vary in different depots.

Another important issue, especially for obese people, is that the classical pharmacokinetic analysis may lead to error in the distribution volume (V_{ss}) estimate. Utilizing classical pharmacokinetic calculations to model highly lipophilic POPs at low concentrations often leads to a substantial underestimation of V_{ss} and mean residence time during the late terminal phase of the elimination time curve. An accurate determination of V_{ss} is required in sound clinical practice because it is critical for the proper selection of a drug treatment regimen or of environmental chemical distribution and kinetics (Berezhkovskiy 2011). Several laboratories use magnetic resonance imaging (MRI) to more accurately study the apparent diffusion coefficient, the distribution of AT in the body, the volume of AT in the different regions, and the different rates of diffusion (Steidle et al. 2011).

When considering POPs as obesogens, it is valuable to revisit the evidence pertaining to their lipophilicity in various tissues. TCDD and dichlorodiphenyltrichloroethane (DDT) are transported out of the gut in the triglyceride phase of chylomicrons (Vost and Maclean 1984). DDT and metabolites also conjugate to hepatic fatty acids, including stearic, oleic, linoleic, and palmitic acids (Leighty et al. 1980). Although several PCBs and organochlorine pesticides in blood are associated with the protein fraction, they are also associated with all major lipoprotein compartments (very low density lipoprotein, VLDL; low density lipoprotein, LDL; high density lipoprotein, HDL) (Vost and Maclean 1984). TCDD was also found in the same lipoprotein compartments of ApoE-/- and wild type mice (Dalton et al. 2001). While these POP-lipid associations are held responsible for their tissue partitioning, they may also be partially responsible for POP lipotoxicity (Leighty et al. 1980).

Recent studies have suggested some heterogeneity exists with respect to both the distribution of POPs across AT depots as well as the association of individual POPs with AT mass. However, studies of the heterogeneity of POP distribution across AT depots need to be confirmed because of the limited number of subjects that were studied (Ronn et al. 2011;

Roos et al. 2012). Some differences in the association of individual POPs with AT mass may be explained by differential lipophilicity of various POPs. For instance, circulating levels of highly chlorinated PCBs have a negative association with AT mass, e.g. PCB-153, -156, -157, -169, -170, -180, -189, -194, -206, and -209, while circulating levels of relatively low chlorine containing PCBs have a positive association with AT mass e.g. PCBs-74, -99, -105 and -118 (Ronn et al. 2011; Roos et al. 2012; Yu et al. 2011). Confirmation of the importance of this heterogeneity may contribute to a better understanding of the relationship between the POP profile in serum and in the AT. The utility of an environmental contamination signature for food contamination evaluation needs further assessment in humans (Antignac et al. 2006).

Clearly, toxicokinetics and computational biology represent important approaches that are needed to understand the interaction of chemicals and AT. Using the recent technical advances, a more quantitative and accurate assessment of these interactions will be possible in the future.

AT AS A SOURCE OF CHRONIC POP EXPOSURE

As mentioned earlier, POPs and other lipophilic xenobiotic chemicals distribute according to their affinity for proteins and lipids and are stored primarily in the AT. They are also found in blood from which they can contaminate other tissues. As discussed below, several observations in both humans and animals suggest that the release of pollutants from AT is an important source of blood POPs.

In humans, most of the evidence has been gathered from studies on drastic weight loss in obese individuals. Such weight loss can be achieved through dietary changes and bariatric surgery and could lead to a decrease exceeding 30 kg of fat mass. Several independent studies have shown an increase in blood POPs following fat loss elicited by dietary changes either alone or coupled with bariatric surgery (Hue et al. 2006; Kim et al. 2011). The role of fat

mass in the control of POP blood levels was further supported by Lim et al. (Lim et al. 2010) who demonstrated an inverse correlation between long-term weight changes and POP serum concentrations.

If increased blood POP levels during weight loss are related to their release from AT, one would expect changes in POP content of AT. This has been addressed by Kim et al. (Kim et al. 2011) who determined POP concentrations in both blood and AT and also assessed the total amount of fat in the studied individuals. The data indicate that POP concentration in AT (expressed per gram lipid) increases with weight loss. While this may seem paradoxical, it is not surprising since the total amount of fat mass decreases considerably, thereby leading to an increased concentration of pollutants in AT. Released POPs can be taken up readily by the remaining fat, which is essentially an infinite sink. Nevertheless, this total POP body burden tends to decrease by 15% following weight loss, at least for certain POPs (Kim et al. 2011). The primary excretion route of most POPs is feces, but may also include maternofetal transfer and lactation, as discussed later (Wendling et al. 1990).

Evidence from wildlife indicates that fasting and AT loss increase circulating POPs. Observational studies were conducted in northern elephant seals which accumulate a large amount of fat in order to cope with fasting that could last several weeks and result in a large amount of AT loss. Debier et al. (Debier et al. 2006) showed that fasting was accompanied by an increase in the serum concentration of PCBs, likely due to release of PCBs from contaminated fat depots. Interestingly, the concentration of PCBs also increased in blubber because of the decreased body fat mass. The mobilization of POPs during fasting may lead to toxic effects.

Experimental evidence also suggests fasting results in redistribution of POPs from their AT storage sites. Indeed, a study shows that in rodents pretreated with hexachlorobenzene, weight loss leads to a time-dependent increase in the brain content of

hexachlorobenzene (Jandacek et al. 2005). In a study where mice were pretreated with DDT, weight loss led to increased DDT in all tissues examined, e.g. brain, lung, heart, spleen, kidney, liver, adipose and blood, except muscle (Ohmiya and Nakai 1977). However, there was no evidence of a change in DDT metabolism or excretion. Thus, decreased AT leads to a redistribution of certain POPs, which favors movement of POPs towards other lipid-rich tissues. The enhanced localization of DDT in the brain was associated with toxic CNS outcomes (Ohmiya and Nakai 1977).

A critical issue is whether the release of POPs from AT observed during weight loss could also lead to toxic outcomes in other organs and tissues of people. Indirect evidence was obtained in humans from several studies of weight loss triggered by either diet or diet associated with bariatric surgery. The Tremblay group showed that increased serum POPs correlated with alterations in resting metabolic rates, thermogenesis, and skeletal muscle oxidative capacity (Imbeault et al. 2002; Pelletier et al. 2002; Tremblay and Chaput 2009). We have shown that whereas all individuals undergoing weight loss improved their blood lipid and liver toxicity parameters, those that had the highest serum POP levels displayed a delayed improvement of these parameters (Kim et al. 2011). This suggests that POPs counteract the positive effects of weight loss on hepatic and serum lipids.

The amount of POPs in breast milk reflects the POP body burden in an individual, and indirect evidence for POP release from fat storage tissue in humans is provided by breastfeeding studies. Many POPs and other xenobiotic chemicals are found in breast milk because of its lipid content. Because of the equilibrium between lipid associated POPs in AT, blood, and milk, it is likely that a significant fraction of breast milk POPs originates from the AT storage compartment, in addition to newly absorbed contaminants. In agreement with this model, AT concentration of DDE in rats at the end of gestation was two to three times greater than after weaning their offspring (You et al. 1999). The apparent half-life of dioxin in

humans is reduced by breastfeeding (Milbrath et al. 2009). While considering the potential negative consequences of POP presence in breast milk, one should keep in mind the important nutritional and immune benefits of breastfeeding.

In conclusion, a number of human and animal studies suggest that AT behaves as a toxicokinetic buffer for lipophilic pollutants (Figure 1). It is a specific storage compartment for these pollutants. However, this is a dynamic situation and release from AT occurs at a low basal level which can be magnified during weight loss. There is indirect evidence suggesting that released POPs exert some toxic effects. More direct evidence for this point is needed.

AT AS A TARGET OF POLLUTANTS

POPs as obesogens. With the obesogen field still in its infancy, experimental research on POPs as obesogens is sparse. We have recently reviewed the literature on developmental exposures that increase risk of obesity, with an emphasis on human exposures, and themes are already emerging (La Merrill and Birnbaum 2011). Development, e.g. prenatal, postnatal, and pubertal, is likely a critical window of susceptibility to obesogen effects of toxic exposures (Figure 2). Programming mechanisms are still unclear (see below), but are believed to involve epigenetic regulation of critical genes that lead to adiposity later in life (Barouki et al. 2012). Evidence suggests that developmental exposures to chemicals that increase risk of obesity sometimes operate in a non-monotonic dose-response manner; cachexia may occur at high doses whereas body and/or adipose mass gain occurs at low doses of the same chemical. Further, there may be gender specific effects of developmental toxic exposures that increase the risk of obesity (Tang-Peronard et al. 2011). Here, we focus on the experimental research on POPs that cause obesity and dyslipidemia. Developmental exposures to these same POPs are positively associated with obesity in humans (Valvi et al. 2012).

Some rodent models indicate that dioxin-like (DL) chemicals may be obesogens. Exposure to 100 μg TCDD/kg b.w. once every 2 weeks for 8 weeks increased body weights of adult mice over 40% higher than control-treated C3H/HeN mice (Zhu et al. 2008). This body weight change was only seen when mice were fed a high fat diet, which was not out of the range of an American diet. In a one-month study, chronic developmental exposure to the PCB mixture Aroclor 1254 was associated with increased body weights of mouse pups on postnatal days (PND) 16-20 (Branchi et al. 2002). Further, adult mice exposed to 49 mg of DL-PCB-77/kg body weight had an AhR dependent increase in body mass (Arsenescu et al. 2008). This PCB-77 exposure also increased body mass, fatty liver, abdominal fat, and adipocyte hypertrophy in CVD model mice (Hennig et al. 2005). Fatty liver, attributed to increased hepatic triglycerides and cholesterol, was also caused by 50 mg of DL-PCB-169/kg body weight (Kohli et al. 1979).

There is limited evidence of increased adiposity in animal studies of POPs that are not DL, however body fat is seldom assessed in studies reporting no increased body mass after POP exposure (La Merrill and Birnbaum 2011). Prenatal exposure to a major polybrominated- diethyl ether (BDE-99, 2,2',4,4',5-penta-BDE) increased mouse birth weight (Lilienthal et al. 2006), and pre- and postnatal exposure to BDE-47 (2,2',4,4'-tetra-BDE) increased rat body weights from birth to puberty (when the study ended) (Suvorov et al. 2009). In the longest study of developmental PBDE exposure to examine body weights, male mice exposed to BDE47 10 days after birth had increased body weights from PND 47 until the end of the study, at 4 months of age (Gee and Moser 2008). These studies all indicate significant body composition effects of perinatal exposure to PBDEs, however the mechanisms remain unclear and the data should be interpreted with caution as certain preparations of BDEs could be contaminated with DL chemicals. In perinatal exposure to perfluorooctanoic acid (PFOA), obesogenic effects do not appear until later in life. Mice

exposed to low levels of PFOA *in utero* had increased body mass once mature, with an inverted U shape dose response curve (Hines et al. 2009). By 18 months of age, there was no longer an effect on mouse weight, however, there was a positive dose response relationship between *in utero* PFOA exposure levels and abdominal brown AT mass in the aged mice, whereas a negative relationship was found with white AT mass. Consistent with experimental findings, a recent prospective human study demonstrated that maternal PFOA levels during pregnancy were associated with obesity in the daughters 20 years later (Halldorsson et al. 2012). Organochlorine pesticides may also increase adiposity. For instance, low doses of lindane elevated the body weights of dogs (Rivett et al. 1978). Likewise, oral DDT exposure increased the body weights of female mice and their offspring in a two- generation chronic exposure study (Tomatis et al. 1972).

Evidence and implications of lipotoxicity. The accumulation of lipids in non-AT tissues has toxic effects on tissue function, and this lipotoxicity may lead to diabetes, hypertension, and heart disease. Many of the POPs that associate with lipids disrupt their homeostasis. Dioxin and DL-PCBs- induced lipotoxicity and dyslipidemia occur even in the absence of an obese phenotype. For instance, PCB-77 elevated serum VLDL in ApoE-/- mice (Arsenescu et al. 2008; Dalton et al. 2001). Similarly, TCDD caused an AhR-dependent increase in the cholesterol content of atherosclerotic plaques and elevated serum LDL in ApoE-/- mice (Arsenescu et al. 2008; Dalton et al. 2001; Wu et al. 2011). The AhR appears to have an innate role in lipid homeostasis. The AhR is activated by LDL and AhR knockout mice have higher levels of serum LDL (McMillan and Bradfield 2007). Further, AhR knockout *C. elegans* larva have elevated fatty acids (Aarnio et al. 2010). There is also evidence to suggest that aryl hydrocarbon receptor nuclear translocator (ARNT, which forms a heterodimer with AhR) activity is required for lipogenesis and glycolysis (Pillai et al. 2011; Wang et al. 2009).

Experimental organochlorine pesticide exposures cause systemic lipotoxicity. DDT exposure increased cholesterol and triglycerides in both serum and AT (Sanyal et al. 1982), and increased hepatic triglyceride synthesis (Sanyal et al. 1982). Similarly, increased triglyceride synthesis was observed in dieldrin exposed rats (Bhatia and Venkitasubramanian 1972). Hepatic fatty acid composition and utilization was also altered when DDT, endosulfan, or dieldrin were administered to rats (Kohli et al. 1975; Narayan et al. 1990).

Lipotoxicity has also been observed with exposure to brominated flame retardants and perfluorinated chemicals. Both male and female rats exposed to a commercial penta-BDE mixture exhibited a dose- related increase in plasma cholesterol (van der Ven et al. 2008). In another study of rats exposed to a commercial penta-BDE mixture, lipolysis rates were increased in *ex vivo* adipocytes (Hoppe and Carey 2007). However, low doses of PFOA reduced total cholesterol and triglycerides in adult rats and had no effect on cholesterol while increasing triglycerides in mice (Loveless et al. 2006). Although the lipid lowering effect of PFOA exposure in these rodent studies is consistent with peroxisome proliferator activated receptor (PPAR)α agonism (Klaunig et al. 2003), PFOA is consistently associated with elevated cholesterol in humans (Steenland et al. 2010). Results of PFOA and PFOS exposures in *PPARα* knock out mice have found gene expression changes indicative of lipotoxicity (Rosen et al. 2008; Rosen et al. 2010) and altered fatty acid metabolism (Rosen et al. 2008). Similarly, PFOS-exposed mice had altered gene expression associated with lipid metabolism (Rosen et al. 2010).

These experimental findings have important implications for epidemiology studies: POP levels in a given tissue are often normalized to lipid content of that tissue. Under the assumption that the total body burden of POPs is evenly distributed in all lipid stores, one can easily compare POP concentrations in different matrices. Unfortunately, the assumption of even distribution of POPs is not always valid. In addition, the correlation and attributable

variation of POPs to lipids varies across studies (Guo et al. 1987; Porta et al. 2009), which is partially due to variation in the lipid extraction methods used by investigators. If some POPs cause both obesity and dyslipidemia through a common causal pathway, normalizing POPs to lipids may inadvertently adjust the effect of POPs towards the null. Indeed a recent longitudinal epidemiology study found weaker, but still significant, associations between POPs and obesity when adjusting for serum triglycerides and cholesterol, suggesting that analysis of lipid-adjusted POPs may represent an over-adjustment, given that these chemicals may also perturb lipid metabolism (Lee et al. 2011). In the absence of definitive information about the causal pathway of the effects of POPs on outcomes for which dyslipidemia may be on the causal pathway (including obesity, diabetes and CVD, and the cancers for which obesity and diabetes increase risk, such as breast cancer), we recommend presenting analyses of POPs both with and without lipid adjustment which is supported by others scientists (Porta et al. 2009).

Disruption of AT function and adipocyte differentiation. The mechanisms through which POPs could induce the disruption of AT function, metabolism, and adipose cell differentiation are diverse. However, because of space limitations, only a few issues will be discussed here. *In vitro* studies are consistent with a positive role of POPs in the risk of obesity. Additionally, POPs can act by altering the activity of metabolic enzymes. For instance, both TCDD and PCB-77 reduced lipoprotein lipase (LPL) activity *in vitro* unless AhR antagonists were present (Hegele 2009; Olsen et al. 1998). *LPL* mutations are associated with severe hyperlipidemia in humans.

POPs can also alter adipocyte differentiation, however the literature in this field is somewhat contradictory. It was shown that low doses of TCDD and PCB-77 could induce adipocyte differentiation *in vitro*, with greater potency of PCB-77 than its toxic equivalency

factor (TEF) would suggest (Arsenescu et al. 2008). In different studies, it was shown that overexpression of the AhR decreased adipocyte differentiation and PPARγ expression, a marker of adipocyte terminal differentiation (Cho et al. 2005; Tontonoz and Spiegelman 2008). Additional evidence suggests multiple and often antagonistic interactions between the AhR and the PPARγ pathways (Remillard and Bunce 2002). Other mechanisms have also been suggested to account for the effect of DL chemicals on adipocyte differentiation: these include the interaction with hormonal or retinoic acid receptors or through the regulation of CCAAT/enhancer binding protein (C/EBP) protein family isoforms (Mullerova and Kopecky 2007; Vogel et al. 2004). In certain cellular systems, large scale studies suggested cooperative antiadipogenic effects of dioxin and growth factors (Hanlon et al. 2005). It is likely that some of the apparently contradictory data are due to different cellular systems, different developmental stages, different species, and different xenobiotic chemical dose. For example, DL chemicals may promote adipocyte differentiation at low doses and display an opposite effect at higher doses.

Cellular and animal studies have been carried out on additional POPs and also indicate pro-obesogenic effects. PPARγ agonism is commonly associated with most candidate obesogens, including perfluoroalkyls, DDT, organotin, phthalates, and thiazolidinediones (Kopec et al. 2010; La Merrill and Birnbaum 2011). For instance, DDT is capable of inducing dose-dependent adipocyte differentiation through increased PPARγ expression (Moreno-Aliaga and Matsumura 2002). These mechanistic studies suggest complex, multiple and dose-dependent effects of POPs on AT differentiation. Whereas the AhR pathway is clearly implicated, other pathways are also involved, leading to non-monotonic dose-effect relationships. Research should clarify these complex and sometimes contradictory effects in the future.

Because of the importance of the inflammatory phenotype in metabolic diseases, one possible action of POPs would be to induce AT inflammation. Many POPs are well characterized immunotoxicants. Several studies have shown that POPs increase the expression of inflammatory genes in adipose cells (Arsenescu et al. 2008; Kern et al. 2002; Li et al. 2008). We have recently shown, in a human model of preadipocytes and adipocytes, that the primary effect of TCDD on gene expression was the induction of the inflammatory pathway (Kim et al. 2012). Furthermore, treatment of mice with 10 μg/kg b.w. of TCDD led to increased gene expression of several cytokines as well as other inflammatory mediators in AT, and, importantly, increased the number of macrophages in this tissue (Kim et al. 2012). Interestingly, in obese individuals, increased AT inflammation correlates with increased metabolic disruption such as insulin resistance and diabetes. These observations suggest that, in addition to their effects on obesity, POPs may contribute to AT inflammation, thereby increasing the likelihood of metabolic disruption (Figure 2).

The mechanisms of DL chemical regulation of inflammation are complex and may depend on the system that is studied. Both anti-inflammatory and pro-inflammatory effects have been described. Because of the endogenous role of the AhR in the regulation of immunity, exogenous AhR ligands could either mimic or disrupt these pathways thereby influencing the regulation of inflammatory gene expression (Esser et al. 2009) (Figure 3). In addition, there are complex interactions between the AhR and critical transcription factors involved in the regulation of inflammation such as nuclear factor of kappa light polypeptide gene enhancer in B-cells (NFκB) (Tian 2009; Vondracek et al. 2011). These interactions can also be observed in the absence of xenobiotics. For instance, the AhR forms a complex with signal transducer and activator of transcription 1 (STAT1) and NF-kB to negatively regulate the innate inflammatory response even in the absence of an exogenous ligand (Kimura et al. 2009) (Figure 3). The interactions of the AhR with nuclear factor, erythroid derived 2, like 2

(NFE2L2) signaling could also account for its regulation of inflammation both in adipocytes and in other cells (Haarmann-Stemmann et al. 2012; Shin et al. 2007). In conclusion, the AhR and its ligands clearly modulate the inflammatory response. These effects could be due to the perturbation of an endogenous function of this receptor as well as to additional effects triggered by xenobiotic activation.

CONCLUSION AND PERSPECTIVES

The studies discussed in this review indicate that AT plays a central role in POP toxicology. This role is complex and may seem paradoxical. Indeed, there is evidence that AT is protective under conditions of acute or subacute exposure to POPs. Storage in the lipid droplets has a buffering effect and prevents the persistence of high blood levels of these POPs and of high POP exposure of other more sensitive lipophilic tissues such as brain.

Furthermore, it is presently unclear where and how POPs are stored within the lipid droplet, whether associations between POPs and lipids alter lipid dynamics, and whether the adipocyte or other AT cells are exposed to higher concentrations of POPs because of increased tissues residence time. The latter point suggests that the storage function of AT leads to increased toxicity of POPs towards this tissue. If confirmed, this would indicate that the effects of POPs on metabolic diseases such as diabetes could be explained by primary toxicity to AT, including inflammation, disruption of metabolism, and altered differentiation. Another likely consequence of the POP-storage function of AT is that this tissue constitutes an internal source of low grade chronic exposure of the organism to POPs. This is best illustrated by the drastic weight loss studies which indicate that a release of POPs into the bloodstream takes place and that this release is associated with metabolic and liver toxicity (Kim et al. 2011).

There is ample evidence that AT is also a direct or indirect target of POP toxicity. The obesogen concept, which highlights the vulnerability of the fetal and childhood periods of life in which tissue and organ development take place, suggests that AT development could be a specific target of POP exposure (Barouki et al. 2012). It is unclear whether increased adiposity is a direct effect, as suggested by *in vitro* studies, or an indirect effect mediated by metabolic disruption elicited by certain POPs. There are a number of possible explanations for the obesogenic effects of POPs. It is tempting however to link these effects to the storage function of AT and to consider increased adipose mass as a long term adaptive response to exposure to POPs. It is also intriguing to note that both exposures to POPs and to nutritional imbalance disrupt metabolic programming leading to obesity and metabolic diseases. Whether these have similar mechanisms is a matter for further study. It is also important to stress that POPs not only display quantitative effects on AT (increased fat mass), but they also alter AT quality, notably through inflammation (Figure 2). These alterations are known to increase the risk of obesity.

There are still several unanswered questions that need to be addressed.

1) Obesogens and epigenetics. The obesogen concept needs to be supported by relevant mechanisms of action. To date, epigenetic alterations appear to be the most likely mechanisms that could explain perinatal programming leading to later life obesity and metabolic diseases (Barouki et al. 2012). Although several POPs have been shown to elicit modifications in DNA methylation or miRNA expression (Baccarelli et al. 2009; Manikkam et al. 2012; McClure et al. 2011), it is still unclear whether such alterations are directly implicated in the obesogenic effect. Research should primarily focus on these issues. It is also important to assess the effects of POPs on stem cells and to identify the most relevant *in vitro* systems. Clearly the validation of a predictive *in vitro* system to test putative obesogenic compounds is an important target for future research.

2) POP location and dynamics. Additional studies should assess the actual localization of POPs within the adipose cell and lipid droplet and the dynamics of POPs following their storage in the AT and following weight loss. These studies should also account for heterogeneity of POP distribution that is dependent on variation both within and between the class of POPs as well as on their physiochemical properties.

3) Mechanisms of action. Experimental studies should attempt to identify the mechanisms involved in POP action on the AT. In many cases, these mechanisms are somewhat contradictory (for example both proinflammatory and anti-inflammatory effects). Understanding these issues is critical. They may be related to dose, to cellular target, and physiological context, etc. The presence of multiple mechanisms could explain non monotonic dose response curves.

4) Endogenous functions. The possible involvement of the AhR, as well as other target receptors, in endogenous functions suggest that xenobiotic ligands may both disrupt these endogenous functions and/or lead to additional toxic effects. Delineation of these effects *in vitro* and *in vivo* is critical to improve our knowledge in this field.

5) Human studies. Biomonitoring of POPs within clinical and epidemiological studies is critical to validate experimental observations reviewed here and to support public health action. Prospective longitudinal studies are the most useful tools in establishing causal relationships with POP exposures. In addition, investigations including a more detailed characterization of exposures (especially perinatal exposures) are invaluable to help identify obesogens and metabolic disruptors.

Overall, AT appears to be a major player in the toxicological responses to POP exposure, both in terms of adaptation and in terms of toxic effects. We hope that the toxicological community will give further attention to this important tissue when examining the detrimental effects of pollutants and drugs.

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Figure Legends

Figure 1: Role of AT in the toxicokinetics of POPs. AT plays a dual role in the regulation of POP kinetics. 1) Upon exposure to POPs, these lipophilic pollutants are stored in liver and AT. This prevents the action of these pollutants in other sensitive tissues and may be protective to a certain extent. 2) However, it is also known that POPs can be released from their storage site in AT which constitutes a source of low grade internal exposure.

Figure 2: POPs as obesogens and as disruptors of AT structure and function. Strong evidence from both *in vivo* and *in vit*ro studies suggest that POPs can influence the development of AT, particularly at low doses. These programming events take place in the early periods of life (e.g., fetal, neonatal) probably through epigenetic mechanisms and could have an impact on later life diseases. In addition, POPs can alter AT function and structure later in life. This occurs primarily through metabolic disruption and inflammation. These effects favor the development of metabolic diseases.

Figure 3: Major signaling mechanisms involved in the effects of DLC POPs on AT. Most, if not all, of the effects of Dioxin-like Compounds are mediated by the AhR. Only genomic effects are shown. The AhR could directly regulate target genes as a heterodimer with ARNT. Several interactions with transcription factors or nuclear receptors have been described and are shown here. POPs could either trigger these interactions or disrupt existing interactions between the AhR and other signaling factors.

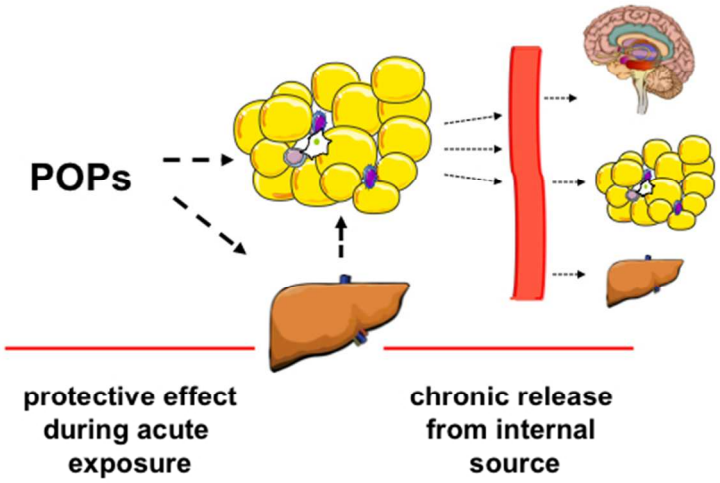
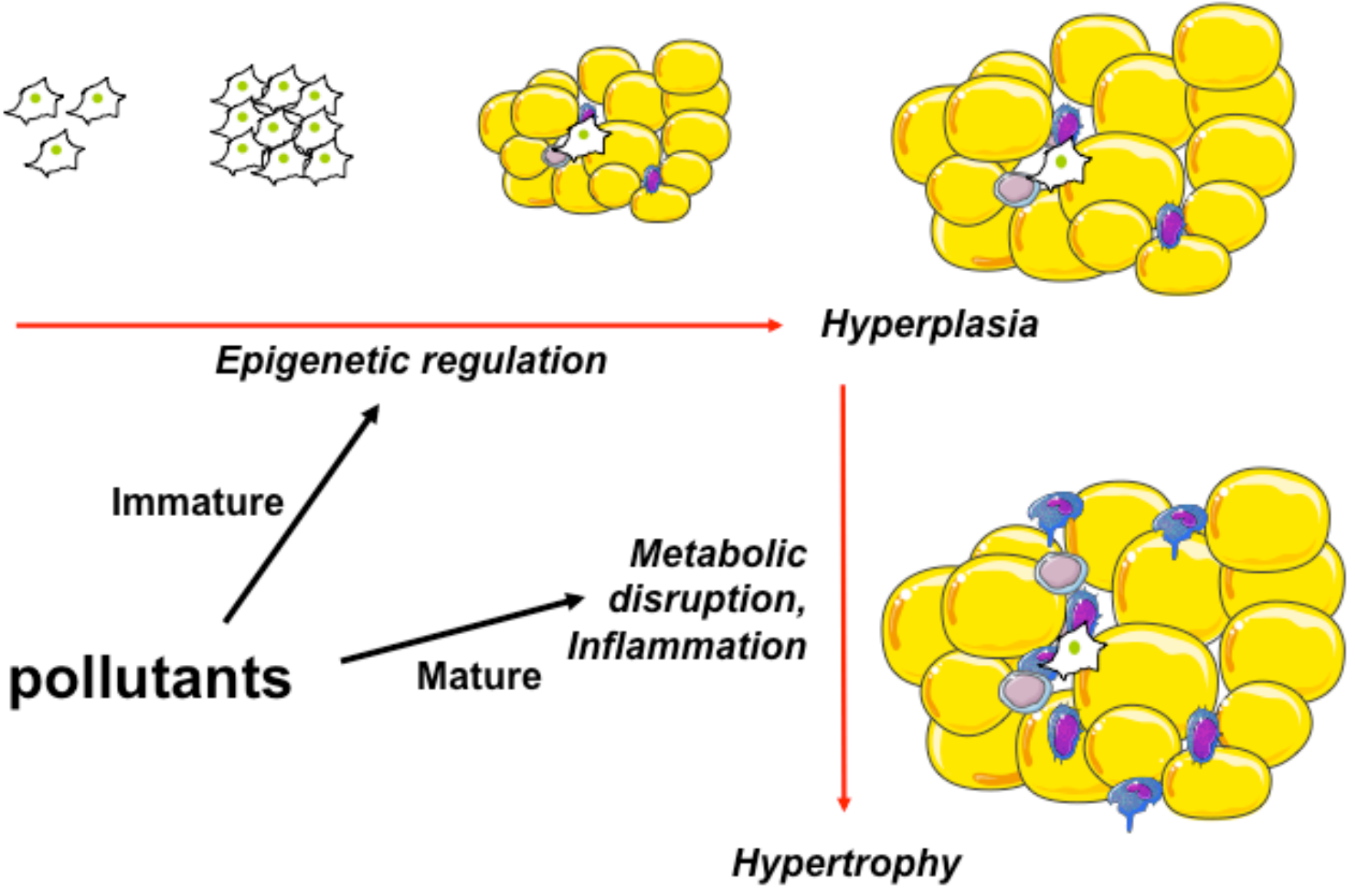
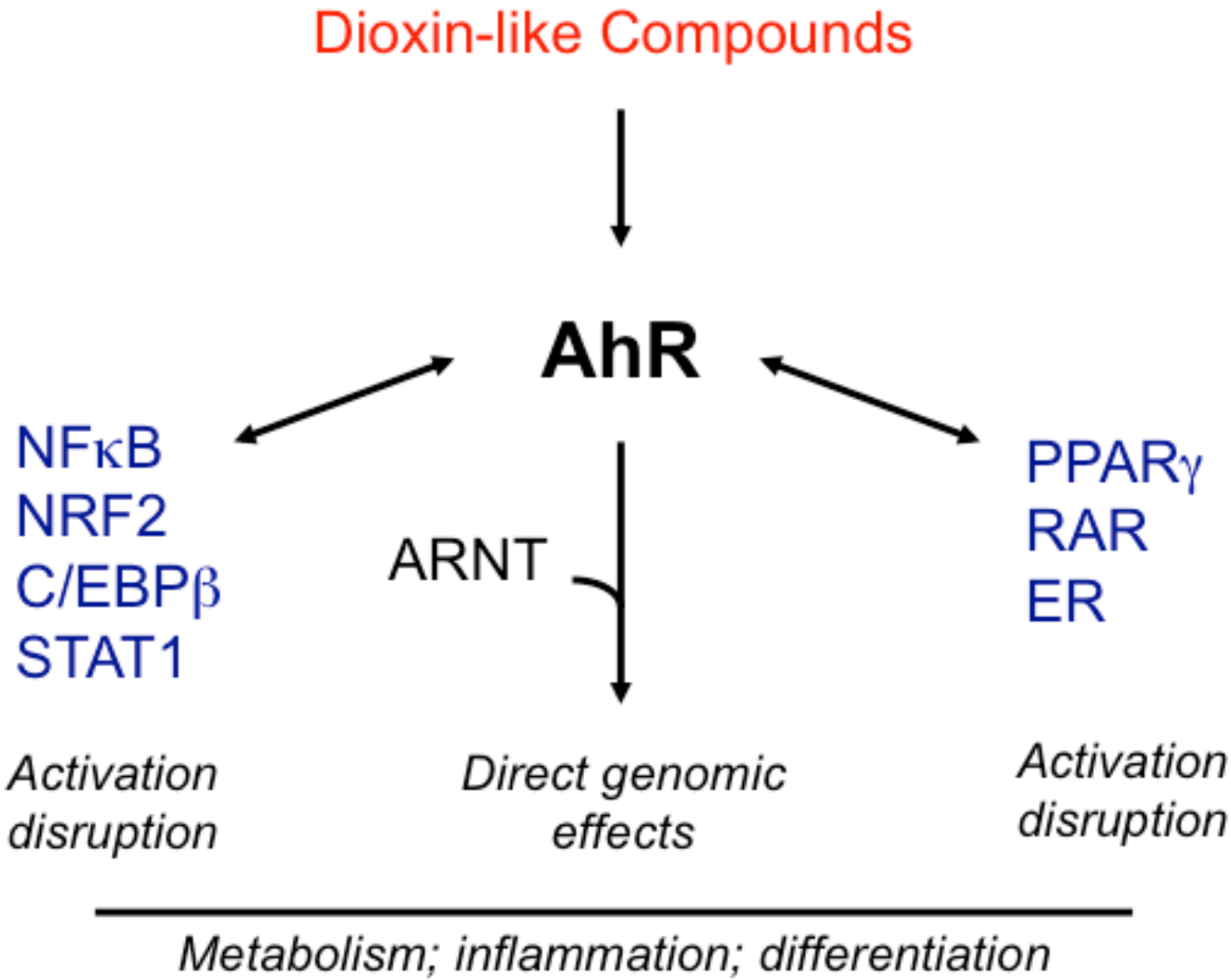


Figure 1

Figure 1
254x190mm (72 x 72 DPI)


Hyperplasia
Epigenetic regulation
Immature
Metabolic
disruption,
Inflammation
pollutants
Mature
Hypertrophy


Dioxin-like Compounds
AhR
NFκB
NRF2
C/EBPβ
STAT1
ARNT
PPARγ
RAR
ER
Activation disruption
Direct genomic effects
Activation disruption
Metabolism; inflammation; differentiation